1: J Biotechnol. 1994 Feb 14;32(2):107-25.

Evaluation of the proteolytic potential of in vitro-cultivated hybridoma and recombinant mammalian cells.

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The proteolytic potential of culture supernatants derived from recombinant baby hamster kidney (BHK) 21 and mouse-mouse hybridoma cells have been characterized. Several assays using enzyme specific chromogenic artificial peptides, as well as a radioactive test for the detection of the total activity, have been established and were adapted to the special conditions existing in culture media of mammalian cells. Proteolytic activity was detected in human serum albumin containing media which was specific for peptides ending with a terminal arginine. The addition of N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer to the culture media resulted in a significant peptide cleavage potential, supporting the fact that this compound is not recommended as a supplement in animal cell culture media. Medium shock protease activity has been detected in culture supernatants of BHK cells when medium was changed completely, caused by a switch from a serum containing state of growth to a serum-free state of growth which is often used in processes with microcarriers. However, this proteolytic activity showed a transient behaviour whereby its secretion stopped when the cells had adapted to the serum-free medium conditions. Characterization of the proteolytic activities using different specific inhibitors and activators supported the assumption that the proteolytic activity reflects a cell specific composition of proteases which can also change dependent on the culture conditions used.

PMID: 7764558 [PubMed - indexed for MEDLINE]

1: Dev Biol Stand. 1987;66:403-8.

Links

Proteolytic activity in the culture supernatants of mouse hybridoma cells.

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When serum-reduced or serum-free culture supernatants were incubated for 16 hours at 37 degrees C, more than 70-80% of original proteins were digested as measured by gel electrophoretic analysis. The proteolytic activity, which was only observed at pH values lower than 4.5, was reduced in conditioned medium containing higher concentrations of fetal calf serum. During incubation large amounts of the monoclonal antibody (IgG1) were cleaved giving F(ab')2 fragments. The results reported here strongly suggest that the conditioned medium of mouse hybridoma cultures contain probably two cellular (acid) proteases with apparent MW of 45-50 K and 90-95 K, respectively. The similarities with the lysosomal cathepsin D are discussed.

PMID: 3556271 [PubMed - indexed for MEDLINE]

1: J Biotechnol. 1991 Oct;20(3):249-61.

Links

Monitoring of the production of monoclonal antibodies by hybridomas. Part II: Characterization and purification of acid proteases present in cell culture supernatant.

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An acid proteolytic activity has been found in cell culture supernatants from long-term cultivations of hybridoma cells in hollow fibre bioreactors using serum free medium. The proteolytic activity has now been further characterized and the main results were: (1) the proteolytic activity showed a maximum around pH 3 and declined essentially to zero at pH 8; (2) the activity was specifically inhibited by pepstatin A; (3) the acid proteases consisted of two sets of closely spaced bands with apparent molecular weights of 40-45K and 90-105K, respectively; (4) the protease bands (40-45K and 90-105K) were reactive with anti-human cathepsin D; (5) the IEP values of the acid proteases ranged from pH 4.55-6.5. Furthermore, IgG incubation with the acid proteases isolated from hybridoma cells yielded fragments similar to those found in serum-free hollow fibre cell culture supernatants. These results indicated that the IgG fragments are the result of degradation by cathepsin D like proteases released after cell death or cell lysis.

PMID: 1367572 [PubMed - indexed for MEDLINE]